

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



AE

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C12Q 1/68, G06F 17/30 // 159:00

A1

(11) International Publication Number: WO 96/23078

(43) International Publication Date: 1 August 1996 (01.08.96)

(21) International Application Number: PCT/US95/12429

(22) International Filing Date: 6 September 1995 (06.09.95)

(30) Priority Data:
PCT/US95/01160 27 January 1995 (27.01.95) WO

(34) Countries for which the regional or
international application was filed: US et al.

(71) Applicant (for all designated States except US): INCYTE
PHARMACEUTICALS, INC. [US/US]; 3174 Porter Drive,
Palo Alto, CA 94304 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): SEILHAMER, Jeffrey, J.
[US/US]; 12555 La Cresta, Los Altos Hills, CA 94022 (US).
DELEGEANE, Angelo [US/US]; 30474 Oakmont Way 1,
Hayward, CA 94544 (US). SCOTT, Randal, W. [US/US];
13140 Sun-Mor, Mountain View, CA 94040 (US).

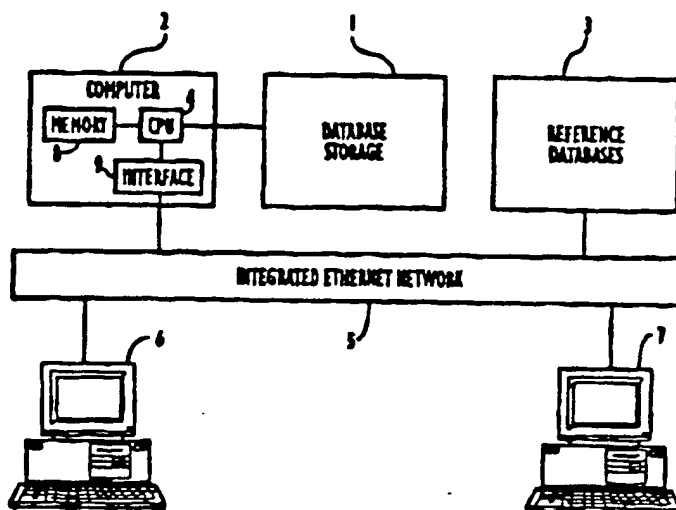
(74) Agents: CAGE, Kenneth, L. et al.; William Brinks Hofer Gilson
& Lione, Suite 200, 2000 K Street, N.W., Washington, DC
20006-1809 (US).

(81) Designated States: AM, AU, BB, BG, BR, BY, CA, CN, CZ,
EE, FI, GE, HU, IS, JP, KG, KP, KR, KZ, LK, LR, LT,
LV, MD, MG, MN, NO, NZ, PL, RO, RU, SG, SI, SK, TJ,
TM, TT, UA, US, UZ, VN, ARIPO patent (KE, MW, SD,
SZ, UG), European patent (AT, BE, CH, DE, DK, ES, FR,
GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF,
BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published

With international search report.

(54) Title: COMPUTER SYSTEM STORING AND ANALYZING MICROBIOLOGICAL DATA



(57) Abstract

[A relational database (1) for the storage of microbiological information] The database contains cDNA sequencing data (290, 300) and corresponding match logs (510, 515) indicating a correlation between presently identified cDNA sequences and previously known sequences. In addition, a variety of the tables that make up the database are used to store historical data related to the identification of a particular cDNA sequence. Such tables include biological source data (130); cell culture and treatment data (140); mRNA preparation data (150); cDNA construction data (170); and clone preparation data. The clone preparation data further includes tables containing data relevant to inoculation (200), preparation (210), excision (190), and a fluorometer (220, 230, 240). The interrelated information in the database allows various queries to be used to extract data for scientific analysis and other applications. For example an abundance analysis may be performed by using the database of the preferred embodiment to determine the frequency a particular RNA transcript appears in a certain source tissue.

AN

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgyzstan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	KZ	Kazakhstan	SG	Singapore
CH	Switzerland	LI	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovakia
CM	Cameroon	LR	Liberia	SN	Senegal
CN	China	LT	Lithuania	SZ	Swaziland
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	LV	Latvia	TG	Togo
DE	Germany	MC	Monaco	TJ	Tajikistan
DK	Denmark	MD	Republic of Moldova	TT	Trinidad and Tobago
EE	Estonia	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	UG	Uganda
FI	Finland	MN	Mongolia	US	United States of America
FR	France	MR	Mauritania	UZ	Uzbekistan
GA	Gabon			VN	Viet Nam

COMPUTER SYSTEM STORING AND ANALYZING MICROBIOLOGICAL DATA

FIELD OF INVENTION

This invention relates to computer database technology applied to genetic data and corresponding cell information. More specifically, a relational database system that stores
5 DNA sequences, the corresponding source data, and other related scientific data is disclosed.

BACKGROUND OF THE INVENTION

Relational databases are generally known in the art. See for example C.J. Date, "An Introduction To Database Systems"
10 Addison-Wesley Publishing Company, 1982 (particularly, Part 2).

In general, a relational database can be characterized as a system for storing data represented as a plurality of tables. A row of each table, also referred to as a tuple,
15 represents a record of information. A column is essentially a collection of values for the same field of the stored records. Each column is also referred to as an attribute of the stored records. In other words, each record in a given table of a relational database includes a set of fields that correspond
20 to the attributes of the table. A set of all the values from which the actual values of an attribute can be drawn is referred to as a domain. As discussed on page 65 of the above-referenced text, "a crucial feature of relational data structure is that associations between tuples (rows) are
25 represented solely by data values in columns drawn from a common domain."

Previously most of the analysis of genetic information has been done using chemical methods in a laboratory. Computerized research tools have been limited essentially to
30 performing comparisons of sequence information to determine whether a particular genetic sequence has been previously identified. Such tools may provide effective searching techniques for genetic sequences; however, they do not store and manipulate diverse scientific information, such as the
35 correlation between the cDNA sequences and the types of cells from which they were derived. Thus, the existing computerized

tools have only a very limited use in the field of diagnostics and drug development research. Presently, there is a pressing need to develop a computer system which stores genetic data and related cell information in a well organized form so as to
5 enable scientists to analyze such data efficiently.

SUMMARY OF THE INVENTION

In accordance with the present invention a relational database for storing biological information is provided. The relational database is organized as a collection of tables
10 each of which stores specific records of biological information. The records are interrelated so that each table includes a column which is common with at least one other table. This enables database queries that can search the database essentially on any attribute of any table.

15 In a preferred embodiment of the invention, the database contains cDNA sequencing data and corresponding match logs indicating the correlation between presently identified cDNA sequences and previously known sequences. In addition, a variety of tables of the database store historical data
20 related to identification of a particular cDNA sequence. Such tables include the identification of the biological source; cell culture and treatment data; mRNA preparation data; cDNA construction data; clone preparation data including tables for inoculation, preparation, fluorometer data, and excision.

25 The interrelated information in the database enables the design of various queries useful in scientific analysis and other applications. For example, such functions as abundance analysis which allows one to determine the frequency with which an RNA transcript appears within a certain source tissue
30 can be performed using database of the preferred embodiment. Other analytical results that have previously been obtained using laboratory chemical techniques can be determined using database queries. One such application is subtraction analysis.

BRIEF DESCRIPTION OF THE DRAWINGS

35 The foregoing features of the present invention may be fully understood from the following detailed disclosure of a

specific preferred embodiment in conjunction with the accompanying drawings in which:

Fig. 1 symbolically depicts an overall architecture of the system of the preferred embodiment of the present invention.

Fig. 2 is a flowchart symbolically depicting the process of cloning and sequencing cDNAs.

Figs. 3A, 3B and 4-10 illustrate portions of the biological relational database of the preferred embodiment of the present invention.

Fig. 11 illustrates an example of the output of an abundance analysis query of the relational database of the preferred embodiment.

Fig. 12 illustrates an example of the output of a subtraction analysis query using the database of the preferred embodiment.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

According to the preferred embodiment of the present invention, a system for storing, tracking and manipulating the genetic data is organized as a relational database. As illustrated in Fig. 1, the users of the system at their workstations (6 and 7) can access one or more relational databases via an integrated Ethernet network 5. The workstations (6, 7) are typically personal computers known in the art that usually include data entry means, output devices, display, CPU, memory (RAM and ROM) and interfaces to network 5. Database storage 1 illustrates the database of the preferred embodiment of the present invention, which is stored at a file server connected to network 5. As illustrated, it is supported by computer 2, which, as known in the art, usually includes CPU 4, data storage means 8, interfaces to the network 9, and input and output devices (not shown). Reference databases 3 illustrate sources of data which, for example, may be searched as part of the use of database 1. Such databases may, for example, include other sequence, nucleic acid, protein, and motif databases.

It is well known that each cell in an organism such as the human body, contains a complete set of genes or genetic information. These genes are either active or inactive at different times in the cell's life cycle. Some genes are
5 active in all cells and are necessary for normal and common functions, or housekeeping duties. Other genes are only active in a particular cell type, because they specify and regulate functions peculiar to a tissue or an organ under normal conditions. Finally, there are genes which are
10 activated only in response to stress or disease. Some stress genes, which activate in several cell types, respond to the general alarm. Other stress genes are very specific and only activate in a particular cell type. Thus genes can be grouped into very small and specific subsets or subsets of varying,
15 larger sizes. The classification and understanding of these nested sets of genes are important in the diagnosis and treatment of disease.

Genes, or double-stranded deoxyribonucleic acid (DNA), are activated by the transcription or copying of the sense
20 strand of the DNA molecule into single-stranded messenger ribonucleic acid (mRNA). The message inherent in the mRNA sequence is subsequently translated into amino acids, the molecular building blocks of the polypeptides or proteins that function structurally or enzymatically in the cell.

25 The activities taking place at any one time and the relative importance of those activities are reflected in the numbers of mRNA molecules found in the cell. Some mRNAs (housekeeping) are always present, and their numbers remain fairly stable in normal cells of any tissue. These mRNAs (eg.
30 actin) represent and carry out the constant background activity essential to most cell types (the exception to this case is a mature, differentiated red blood cell which lacks DNA but has a set of mRNAs or enzymes which function for the remainder of its life). In contrast, the RNAs (routine) which
35 carry out the duties of a particular cell type are only activated in that cell type, and the numbers of routine mRNAs will be stable under normal conditions. If that particular

cell type is stressed or exposed to disease, the numbers of routine mRNAs fluctuate as genes which respond to the stress/disease are activated. These stress/disease mRNAs have priority over other routine or housekeeping mRNAs, and they quickly increase in number.

For example, the housekeeping genes of brain cells and liver cells are shared; cells from both organs transcribe the mRNAs that produce the enzymes required to process incoming molecules of glucose. However, the mRNAs that make proteins for the normal functions of a pituitary cell are different from the mRNAs of a liver Kupffer cell although each is functioning normally. Likewise, the set of mRNAs from a diseased liver cell differ from those from a normal liver cell. In each case, a different and diverse subset of mRNAs characterizes the cell in a particular situation at a particular time.

The database of the preferred embodiment provides the storage, manipulation, and retrieval of the information which relates to the classification and characterization of unique populations of mRNAs. On the basis of this information, scientists can diagnose diseases and design specific treatments. The wealth of detailed information provides clues to earlier diagnosis and treatment which contribute to rapid healing and help avoid permanent impairment or death.

The database system of the present invention takes advantage of the powerful capabilities of modern computers by storing genetic information in association with a large amount of related information. More specifically, in the preferred embodiment, the information on essentially all the steps of obtaining tissue, extracting transcripts, cloning, and identifying cDNA sequences is stored in various relational tables. Thus, the database of the present invention allows one to backtrack through the steps performed in the laboratory in identifying the cDNA sequence. The diverse data stored in the system of the present invention will in many instances answer questions frequently asked in molecular biology and pharmacology without requiring actual experiments, such as:

What are the most active genes and common functions of a particular cell type? What happens to housekeeping and cell-specific routine functions during stress or disease? What genes are diagnostic of the normal or disease state? Which
5 gene products are targets for pharmaceutical intervention?

Fig. 2 illustrates the steps of preparing genetic data stored in the database of the present invention. The information associated with the steps of Fig. 2 is stored in the database as tables depicted in Figs. 3A, 3B and 4 through
10 10. In Fig. 2 the first step 10 is cell preparation. Cell preparation 10 includes the steps of obtaining and growing the cells so as to prepare them for RNA extraction. The following step 20 indicates the processes associated with extracting mRNA from the cell. Next, at step 30, the mRNA
15 becomes cDNA. Alternatively, the cDNA fragment can be received from an outside source or collaborator without performing steps 10 and 20. Once the cDNA molecule is obtained, it is cloned at step 40 and sequenced at step 50. The sequence that is obtained at step 50 is then compared at
20 step 60 to known sequences on the genetic database. Finally, the function of the DNA sequence is determined at step 70.

Figs. 3A, 3B and 4-10 schematically illustrate the tables of the database of the preferred embodiment. Exemplary fields (or attributes) are depicted within each
25 box, and each table includes an attribute having a domain which is common to at least one other table. For example, consider the table indicated as 130 "Biological Source" and the table indicated as 140 "Cell Culture/Treatment". In these two tables the common domain is bio_source_ID. Also,
30 notice that Arrow 135, one end of which is labelled "1" and the other end is labelled "M", indicates that for each one tuple in the Biological Source table there may be more than one tuple in the Cell Culture/Treatment table.

The data received and obtained in steps 10-30 of Fig. 2
35 is stored in the Library Preparation portion of the database of the present invention (Figs. 3A and 3B). This data includes information relating to the biological source of the cells

used to obtain the cDNA (boxes 130, 110, 120), cell culture and treatment (boxes 140, 180), mRNA preparation (box 150) and cDNA construction (boxes 170, 160). More specifically box 130 depicts the table for storing the biological source information. The source may be cells grown in tissue culture or cells obtained during surgery from a single individual or a pooled sample, e.g., pituitary glands obtained from patients of both sexes and a range of ages. In the preferred embodiment the biological source table 130 contains attributes as depicted in Fig. 3A, such as tissue, organ, gender, age, pathology, etc. The biological source may reflect a normal, treated or diseased state. A person skilled in the art will realize that, if desirable, certain other biological source information can be stored; and on the basis of this disclosure, such person will be able to include other relevant attributes if desired.

The data regarding the collaborators, i.e contributors of a biological source, is stored in table 110 as depicted in Fig. 3A, and the information regarding the cell suppliers contributing to biological sources is stored in table 120. The source_ID attribute of the biological source table 130 corresponds to either collaborator_ID or supplier_ID of tables 110 and 120 respectively.

Part of the cell preparation procedure includes the cell culture and treatment process. Cell culture is carried out in containers of known size or volume. Density is usually reported as cells per milliliter (of liquid media) and is monitored to maintain a healthy cell culture. Density at the time cells are harvested may be measured either as cell number or as grams per liter. Treatment may vary. Induction with a chemical can change a cell from an immature form, monocyte, to a mature one, macrophage. Stimulation or activation with a different chemical causes the macrophage to ingest and digest invading bacteria.

In some cases, a cell culture is split into two or more parts, with one subsample maintained in its normal growth mode (as the biological control) and other subsample(s) subjected

to activation and/or stimulation. In a simple scenario, a subsample of control cells is compared with a subsample of cells treated with a drug candidate. Drug doses and length of treatment may vary.

- 5 The cell culture and treatment information is stored in table 140 in Fig. 3A. The attributes of the cell culture/treatment table 140 of the preferred embodiment are listed in the table 140. These attributes include such information as cell density, cell quantity, and treatment.
- 10 The cell culture/treatment table 140 has the attribute `bio_source_ID` in common with table 130. Specific treatment information is stored in table 180 which includes the attributes depicted in Fig. 3A. The `culture_ID` attribute is common to both tables 140 and 180.
- 15 Step 20 of mRNA preparation begins with the extraction of total ribonucleic acid (RNA) from cells of a known weight or volume according to a standard protocol. The protocol and any modifications are recorded. The extracted RNA is optionally fractionated to recover the messenger or
- 20 transcript RNA (mRNA); if it is fractionated then yield is calculated as a percent ($\text{mRNA}/\text{total RNA}$). The normal function of mRNAs in the cell is to produce peptides or proteins.
- Spectrophotometry and gel appearance are used to check
- 25 the quality of the mRNA. In spectrophotometry, an optical density readout of 1.8, derived from a 260 lambda/280 lambda ratio, indicates high quality RNA, not unduly contaminated with DNA or proteins. A subsample of this mRNA is checked further by moving it via electric current (electrophoresis)
- 30 through an agarose gel. The gel is examined visually for contaminating DNA, which generally moves with higher molecular weight substances than the RNA, or for degraded mRNA, which forms a fuzzy rather than a sharp band or signal.
- The data related to the mRNA preparation is stored in
- 35 table 150 in Fig. 3B. Table 150 has an attribute `mRNA_source_ID`, which correlates with either attribute `culture_ID` of table 140 or attribute `Bio_source_ID` of table 130, and an attribute `mRNA_source`, which identifies the table

with which mRNA_source_ID correlates. These two attributes in combination, therefore, link records of table 150 to tables 140 and table 130.

Next, as shown in step 30 of Fig. 2, a cDNA sequence is
5 derived from the mRNA. The cDNA construction requires the conversion of mRNA into complementary DNA (cDNA) preferably using oligo DT, random priming, reverse transcription or other protocols, as known in the art. Useful cloning sites are designed into the bacteriophage into which the DNA is
10 packaged or incorporated. Packaging or plating efficiency is determined by examining the number of primary plaques, i.e., individual bacterial colonies, which resulted from a particular experiment. Information is recorded about the genetic background of host bacterium and the titer of the
15 bacteriophage, before and after amplification. The quality of the library is determined by screening for the actin gene, present in all normal or diseased cell types, and estimation of the size of the cDNA fragment which has been inserted (insert size).

20 The data related to the cDNA construction is stored in table 170 in Fig. 3B. As apparent to a person skilled in the art, the attributes of this table depicted in Fig. 3B provide detailed information about the cDNA construction. Note that tables 170 and 150 have a common attribute mRNA_prep_ID.

25 Preprocessed cDNA fragments can be purchased from an outside supplier or obtained from a collaborator or customer. In such a case, the relevant data is stored in the cDNA supplier table 160 is stored in the database. The Table 160 has the attribute supplier_ID which is also a part of the
30 cDNA construction table 170.

As depicted in Fig. 2, after the cDNA has been constructed, the cloning process, is performed. The portion of the database depicted in Fig. 4 relates to the clone preparation data that is obtained during the cloning process
35 and includes information relating to excision (box 190), inoculation (box 200), preparation (box 210), fluorometer

(boxes 220, 230, 240). Cloning includes the steps of excision, inoculation and preparation.

Excision is the removal of the cDNA fragment from the vector. This follows an overnight cultivation and induced
5 amplification of the vector in the SOLR bacterial host cells which comprise each culture. The plasmid DNA is separated from the bacterial DNA and quantitated fluorometrically before sequencing. The table that stores data related to excision is illustrated as 190 in Fig. 4. The excision table 190 has an
10 attribute cDNA_const_ID in common with cDNA construction table 170.

Inoculation involves growing up or increasing the number of bacteria in a liquid growth medium. As soon as the required cell density (optimum growth) is reached, the culture
15 is plated (streaked or spread thinly) on solid growth media. Individual colonies which arise on the surface of this solid media may be subcultured in tubes or microtiter plate wells of liquid media as pure cultures. The collection of bacterial cultures corresponds to the numbers and type of genes which
20 were active in the source tissue. The data that relates to inoculation is stored in the table illustrated as 200. The attribute plating_ID of the table 200 is common with the same attribute in the table 190.

Fluorometers are used to quantitate the cDNA in nanograms
25 or micrograms per microliter. The total amount of cDNA must be determined to calculate the amount which will be processed and separated electrophoretically in any particular lane of a sequencing gel. The remainder of the sample is stored for future use. Fluorimetry procedures determine cDNA purity and
30 help predict performance in subsequent procedures.

The fluorometer information is stored in the tables illustrated as 220, 230, and 240. More specifically, the data from the fluorometer analysis is stored as the attributes of fluorometer log table 220. Table 230 (Fluorometer) stores the
35 information regarding the instrument and, as illustrated in Fig. 4, has an attribute fluorometer_ID in common with the Table 220. The fluorometer calibration table 240 is

associated with the fluorometer table 230 via a common calibration_ID attribute.

After fluorometry analysis, the cDNAs are prepared for sequencing. Preparation of the cDNAs for sequencing is
5 recorded along with the methods (and their modifications) used at that time. The scientists (SWAT) troubleshoot the sequencing process and track the results of their custom protocols. The preparation table is illustrated as 210.

Table 250, clone log, combines the information regarding
10 the cloning process as illustrated in Fig. 4. In particular, it contains an attribute Inoculation_ID which is also an attribute of the inoculation table 200. An attribute clone_ID is shared with the fluorometer log table 220. An attribute Preparation_ID is also a part of the preparation table 210.
15 The dead_or_alive attribute of the clone log table 250, for example, identifies dead clones in which the plasmid preparation did not yield enough DNA to sequence.

The data related to the process of sequencing is stored as depicted in the sequencing portion of the database
20 illustrated in Fig. 5. This portion includes information relating to specifications of the sequence and related information. It includes the sequencing log (box 300) the sequencing gel (box 280), the reaction set (box 270) and the sequence archive (box 290). The specification of the sequence
25 and related information are stored as attributes in sequencing log table 300. It should be noted that a clone can be sequenced multiple times. Table 260 (sequencing link) links the clone log table 250 with the sequencing log table 300. The sequencing link table 260 contains a clone_ID attribute,
30 which is in common with the same attribute in the clone log table 250 and a sequencing_log_ID attribute which is also included in the table 300.

Sequencing of the cDNAs is performed on an automated ABI system. The sequencing gel is evaluated for the sharpness and
35 darkness of the signal which each of the deoxyribonucleotides or bases (adenine, cytosine, guanine, and thymidine) display, their physical proximity to one another in the gel, and the

clarity of the gel background. These characteristics must fall within certain parameters for the automatic gel reader to produce a sequence. An electronic chromatogram, or gel representation, is stored in the computer system for future
5 reference.

The tracking of all gel information is reflected by a gel key. The gel, the conditions under which it was run, the time required for the gel run, the individual machine/instrument used, staff and biological preparation are recorded whether or
10 not a usable sequence is obtained. This data is stored in the gel key table 280 which has an attribute Gel_key_ID which is common with the same attribute in the sequence log table 300.

The biological preparation, which runs on the sequencing gel, is referred to as the reaction set. The Catalyst is the
15 Model 800 Molecular Biology Station in which robots perform amplifications, PCRs, dilutions and additions of fluorescent dyes to the cDNAs. The data related to the reaction set is stored in table 270. This table has an attribute entitled Reaction_Set_ID which is also part of the sequence log table
20 300.

The sequence archive is activated if a sequence is obtained. The sequence is rated as normal or variant and evaluated for usefulness and subsequent storage in the computer system database. Variant sequences identified at
25 this time may be designated express (see discussion below). The sequence archive data is stored in the table 290 which has the sequence_ID attribute in common with the Sequence Log table 300.

Fig. 6 illustrates a portion of the database for storing
30 information regarding the sequencing equipment. The Sequencer Maintenance Log table 900 collects information on maintenance of each DNA sequencing machine, which via the relational database can be related back to any DNA sequence. The Sequencer Maintenance Log table 900 is linked with the Gel Key
35 table 280 via the common attribute of instrument_number. Table 900 includes such information as the date service was requested, the date service/maintenance was performed, the

nature of the problem, staff involved in maintenance and pertinent comments.

In a preferred embodiment, the Catalyst and Computer Maintenance Logs tables (905 and 910 respectively) are linked through the computer_ID attribute and include similar information to that of the Sequencer Maintenance Log and can be related to essentially any DNA sequence.

The Equipment Log table 915 connects with Maintenance tables 900-910 via the instrument_number and computer_ID attributes and has information on the equipment or instruments used in the sequencing operation. In a preferred embodiment, table 915 stores information regarding equipment name and serial number, vendor identifier, and date installed.

A separate vendor table 920 connects with the Equipment Log Table 915 via the vendor_identifier attribute, and stores, for example, the company name, address, phone number, fax number and contact person. The vendor listing can also have additional information on the vendor, including E-mail address and date contract signed.

Fig. 7 illustrates a portion of the database of the preferred embodiment for storing information regarding the sequencing reagents. The Gel Link table 925 links to the Gel Key table 280 via the gel_key_attribute and to the gel solution table 935 via the gel_solution_ID attribute.

The Gel Solution table 935 includes information on the gel solution and further includes the date the solution was made and who prepared the solution. The Gel Solution-lot Link table 950 links to the gel solution table 935 via the gel_solution_ID attribute and also includes lot_number, and reagent_ID attributes which are shared with the Lot table 965.

The Reaction-Cocktail Link table 930 shares the reaction_set_ID attributes with the reaction set table 270. The Reaction-Cocktail Link table 930 shares cocktail_ID with the Cocktail table 940. The cocktail table 940 also has the date the cocktail was made and staff person who made the cocktail. The Cocktail-Lot link table 955 has the cocktail_ID

attribute in common with the Cocktail table 940 and the Lot-number and Reagent_id in common with the Lot table 965.

The Lot table 965 includes reagent ID and lot number, vendor identifier, date received and date used. The vendor_ID
5 attribute is shared with the Vendor table 960. A separate reagent table 970 shares the Reagent_ID attribute with the Lot table 965 and also has an expanded reagent name.

Experimental sets of sequences may be stored in the database in the express sets portion shown in Fig. 8. This
10 portion includes an express link table 370, a clone variant table 380, an experimental table 390, a clean up table 400 and a resequencing table 410. Express Link table 370 stores sequence sets which have higher priority. They are given unique identifiers and handled separately from the batch
15 process materials. Clone Variant table 380 refers to variant sequences flagged by an individual investigator. The variants are evaluated by that scientist, collaborator, or customer and appropriate action is taken. The experimental sequences stored in Experimental table 390 are similar to the variants
20 above. They may be homologous, allelic or mutant sequences which have been flagged by a particular scientist. If only a fragment has been recovered, a full length expression sequence is ordered, and investigation continued. Cleanup table 400 stores data reflecting the addition of extra steps to the
25 protocol. The longer procedure is designed to improve readability of the sequence. Resequencing is simply repeating the procedure in order to check a sequence or to obtain more data. Information regarding resequencing is stored in Resequencing table 410.

30 Express Link table 370 contains a clone_ID attribute which is also included in the Clone Log table 250. Attribute log_entity_ID of the table 370 provides a correlation with variant_ID, experimental_set_ID, cleanUp_set_ID, and resequencing_set_ID of the tables 380, 390, 400, 410
35 respectively. Log_table_name attribute of the table 370 identifies the table correlated by the Log_entity_ID.

As illustrated in step 60 of Fig. 2, each cDNA sequence that has been obtained in step 50 is then compared to the known sequences in the genetic databases to identify it if possible. This process involves comparing sequences (a) within a data set, (b) within the internal database and/or (c) with external databases. Since the library represents the frequency with which an RNA transcript appears within a certain source tissue, several different clones may contain all or parts of the same gene or its allele(s). The computer also analyzes insert size by counting individual nucleotides in the sequence.

Data relating to sequence comparison is stored in tables on the sequence comparison portion of the database shown in Fig. 7. These tables include a first sequence match log table 510 and a second sequence match log table 515.

The database of the present invention may also access external databases. Genetic databases may have DNA or protein sequences. Such databases services may also provide searching or matching tools in addition to named DNAs, proteins or fragments thereof. As illustrated in Fig. 7, such outside databases include the GenBank database (box 610), the ProDom database (box 570), the Blocks database (box 580), the Pisearch database (box 590) and the Sites database (box 600).

The Genbank database is used as a primary source of known genes, sequences and other information against which the sequencing stored in the database are compared. Percent identity and probability are both considered to determine whether such fragments may be categorized as "exact" (apparently identical to a known/named human sequence), or homologous (partially related) to a gene identified in humans or another species. Unique and unidentified fragments or sequences are listed by an identifier.

ProDom, Blocks, and Pisearch databases may be accessed in order to determine if a particular sequence contains functional protein domains or motifs. The patterns may provide important structural information for a peptide or protein encoded by the sequence.

In addition, Vectors database 520 stores the DNA sequences of the vectors used to clone the cDNAs. By comparing the identified cDNA sequences to the sequences in this database, vector sequences or stretches of vector sequences that show up in a cDNA sequence can be delimited. Similarly, Repeats database 530 allows repeats which belong to a multigene family, such as alu, to be identified. Hidden Markov database 560 contains software which looks at a nucleotide sequence alignment and computes a predicted peptide structure from that sequence. As shown in Box 550 of Fig. 9, other databases which provide additional features can also be accessed.

When a sequence comparison results in a match, the information regarding that match is stored in Sequence Match Log tables 510 and 515. This information generally includes address information for the matching sequence record in the external database as well as scores which represent the quality of the match. In an alternative embodiment it may be preferable to store the scores in a separate record, since the scoring methods are not identical for all databases. Sequence Match Log 510 is linked to sequence archive 290 by the attribute sequence_ID which they share. It should be noted that first Sequence_Match_Log 510 contains better matches, while marginal matches are stored in the second sequence_Match Log 515. Both tables (510 and 515) have identical attributes.

Function identification, illustrated as step 70 in Fig. 2, is then performed on matches whose quality is above a specific threshold. The data related to function identification is stored in the tables as shown in Fig. 10. These tables include a protein table 720, a protein-sequence link table 730, a folder table 760 and location table 780. Protein identification may come from any of the function/domain databases. The Genbank location or locus and the international EC number (enzyme or protein classification) are stored in table 720. Each entry in this table corresponds to one or more sequences from the sequence archive table which was conclusively identified with respect to its function.

Protein table 720 is linked to Sequence Archive table 290 via Protein-Sequence Link table 730. Protein table 720 has the attribute protein_ID in common with Protein-Sequence Link table 730; and Sequence Archive table 290 has the attribute
5 sequence_ID in common with Protein-Sequence Link table 730.

Each entry in folder table 760 contains unstructured annotations for one or more sequences from the archive table which had interesting but inconclusive matches with the other databases. Any type of annotation, footnote, or remark can be
10 recorded in the folder table 760. This permits the researcher to store desired information without contaminating other records in the database with information from inconclusive matches.

Folder table 760 is linked to sequence archive 290 via
15 function sequence link 750. Function sequence link 750 has an attribute Folder_ID in common with folder table 760 and an attribute Sequence_ID in common with sequence archive 290.

The present invention permits a researcher to search the relational database using keywords and to specify the table(s)
20 in which the keyword search should be performed. Thus, for example, a researcher could query the database for all occurrences of the word "endothelial" in the Biological Source Table 130.

In addition, the present invention allows the researcher
25 to store queries in Keywords table 790 shown in Fig. 10. Each query stored in this table is identified by a unique Keyword_ID. When a researcher wishes to run a particular stored query, he or she simply enters the keyword_ID for the query. The computer then pulls up the associated record, and
30 searches the table(s) identified in the Table_name field for the keyword(s) stored in the Keyword_text field. The results of the search can be delivered to the user for example via E-mail notification as shown in boxes 800-820 of Fig. 10.

Location table 780 stores information regarding the
35 location within the cell of each identified sequence. Location table 780 is linked to Protein table 720 by common attribute Protein_ID, and stores the location information in

an attribute called "Location." In a preferred embodiment, the domain for this attribute consists of these categories: nuclear, cytoplasmic (cytoskeleton), cytoplasmic (intracellular membranes), cytoplasmic (mitochondria), cell surface, and secreted.

Also shown in Fig. 10 is GDB links table 770 which links Protein table 720 to the Human Genome Database. GDB links table 770 has attribute Protein_ID in common with Protein table 720 and links to the Human Genome Database via attribute GDB_ID.

Given the wealth of related information stored in the database of the preferred embodiment, a user can now perform new types of data queries not previously available in the known genetic databases. For example, the relational database of the preferred embodiment is well suited for performing abundance analysis. This analysis provides a user with the relative frequency of mRNAs or transcripts found in a particular cell in a given state, e.g., normal or activated. For example, if a researcher were to input a query requesting the most abundant sequences in an LPS activated THP-1 cell, the computer system is programmed to search the relational database and output to the user a display such as, illustrated in Fig. 11.

In the preferred embodiment, the search is performed as follows. First, the cell culture/treatment records in which the cell_line_name field equals "THP-1" (in this example) are identified. Next, the identified records are searched for records in which the treatment field equals "LPS." Then, the sequence match log records correlated in the database with this subset of identified records are determined and the number of sequence match log records for each distinct match ID value is counted to determine the abundance in the cell of the particular sequence identified by the match ID number. After the computer has examined all the biological source records, it sorts the obtained abundance information in the manner requested in the specific query and displays it as a chart, as exemplified in Fig. 11.

Similarly, the database structure described above provides a convenient way to implement subtraction analysis. Subtraction analysis determines which sequences are expressed more commonly in an activated cell compared to a normal cell.

5 To perform subtraction analysis, abundance analysis is performed for the normal cell library and the activated cell library, and when the information is obtained, a ratio of the values is determined. Fig. 12 exemplifies the output of such an operation for normal versus LPS activated THP-1.

10 Location analysis can also be performed. Here, the user requests, for example, the location of a specific protein within a particular activated macrophage. The computer identifies the subset of records associated with the desired cell in the manner described above, consults the associated
15 records in Protein table 720 to verify that the protein is present in the cell, and finally looks up the location of the protein in Location table 780 and outputs the location to the user.

The sequence location table categories in the preferred
20 embodiment are nuclear, cytoplasmic, cell surface or secreted. Within the cytoplasm, sequences may be assigned to cytoskeleton, intracellular membranes, or mitochondria. This information is provided in the location field of Location table 780. All of the unidentified sequences, regardless of
25 their relative abundance, are by default relegated to the unknown category.

Yet another function supported by the database of the preferred embodiment is distribution. This function determines in which tissues or organs for example a given
30 sequence is found and how frequently. The system steps through the records in the Sequencing Log 300 and when there is a match with the desired sequence the system determines the organ and tissue where the specified sequence was found through the relational association of the database. After all
35 the sequences have been examined, an output is prepared representing the requested distribution statistics.

The detailed records and relational structure of the database allow the researcher to access practically any field reflecting a step in the mRNA, cDNA sequencing process. Thus, the database of the present invention provides a powerful tool
5 for analyzing test results as well as testing procedures. For example, if a researcher is interested in knowing all the sequences that resulted from a particular lot or batch of mRNA, this information can be obtained by stepping through the mRNA preparation records 150, finding the records with the
10 desired lot number and outputting the related entries in the sequencing log.

Given the disclosure above, a person skilled in the art can design numerous queries to assist the scientist in various data analysis tasks. From the foregoing description, it is
15 clear that the present invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The presently disclosed embodiments are therefore to be considered in all respects as illustrative and not restrictive, the scope of the invention being
20 indicated by the appended claims rather than the foregoing description, and all changes which come within the meaning and range of equivalency of the claims are therefore intended to be embraced therein.

CLAIMS

1. A computerized storage and retrieval system of biological information comprising data entry means, display means, central processing unit, and data storage means for
5 storing data in a relational data base wherein the database comprises tables, each table having a domain of at least one attribute in common with at least one other table, said tables comprising:
 - a plurality of tables for storing library preparation
10 data;
 - a plurality of tables for storing clone preparation data;
 - a plurality of tables for storing sequencing data; and
 - at least one table for storing sequence comparison data.
2. The database of the system of claim 1 further
15 comprising at least one table for storing functional identification data.
3. The database of the system of claim 1 further comprising tables for storing express sets.
4. The database of the system of claim 1 wherein the
20 tables for storing library preparation data comprise a table for storing mRNA preparation data.
5. The database of the system of claim 1 wherein the tables for storing library preparation data comprise a table for storing cDNA construction data.
- 25 6. The database of the system of claim 1 wherein the tables for storing library preparation data comprise a table for storing biological source data.
7. The database of the system of claim 1 wherein the tables for storing library preparation data comprise a table
30 for storing cell culture and treatment data.
8. The database of the system of claim 1 wherein the tables for storing clone preparation data comprise a table for storing inoculation data.
9. The database of the system of claim 8 wherein the
35 tables for storing clone preparation data comprise a table for storing excision data.

10. The database of the system of claim 9 wherein the tables for storing clone preparation data comprise at least one table for storing fluorometer data.

11. The database of the system of claim 1 wherein the
5 tables for storing sequencing data comprise a sequencing log table.

12. The database of the system of claim 1 wherein the tables for storing sequencing data comprise at least one table for storing reaction set data.

10 13. The database of the system of claim 1 wherein the tables for storing sequencing data comprise at least one table for storing gel key data.

14. The database of the system of claim 2 wherein the tables for storing functional identification data comprises at
15 least one table for storing protein data.

15. The database of system 1, further comprising tables for storing sequencing reagents data.

16. The database of system 1 further comprises tables for storing sequencing equipment data.

20 17. A computer system for storing and retrieving biological data comprising:

a relational database for storing biological data comprising a plurality of interrelated tables wherein each table comprises an attribute having a common domain with an
25 attribute of at least one other table in the database; and means for determining the frequency with which an RNA transcript appears within a certain source tissue on the basis of the data stored in the relational database.

18. A system of claim 17 further comprising means for
30 performing a subtraction analysis of the certain source tissue so as to determine a ratio between the frequency within which an RNA transcript appears within the certain source tissue and the frequency within which an RNA transcript appears in the certain source tissue being in a different state.

35 19. A computer system for storing and retrieving biological data comprising:

a relational database for storing said biological data, said database comprising a plurality of tables each of said tables having at least one attribute having a common domain with an attribute of at least one other table of the database;

5 and

means for determining on the basis of the data stored in the database the location of an mRNA within a given cell.

20. A computer system for storing and retrieving biological data comprising:

10 a database comprising tables wherein said biological information is stored such that the tables are interrelated by having at least one common attribute;

means for determining a presence and frequency of a specific RNA in each of a plurality of organs.

15

FIG. 1

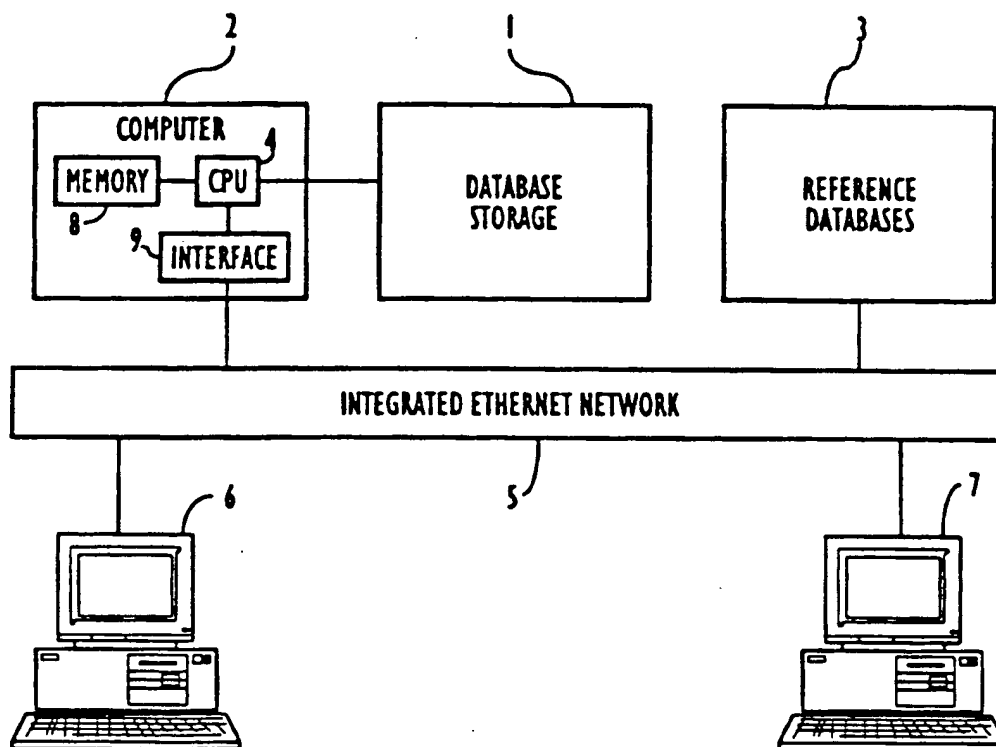


FIG. 2

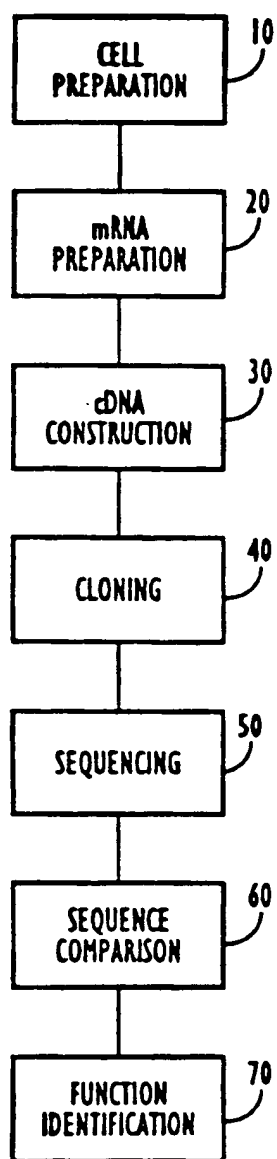
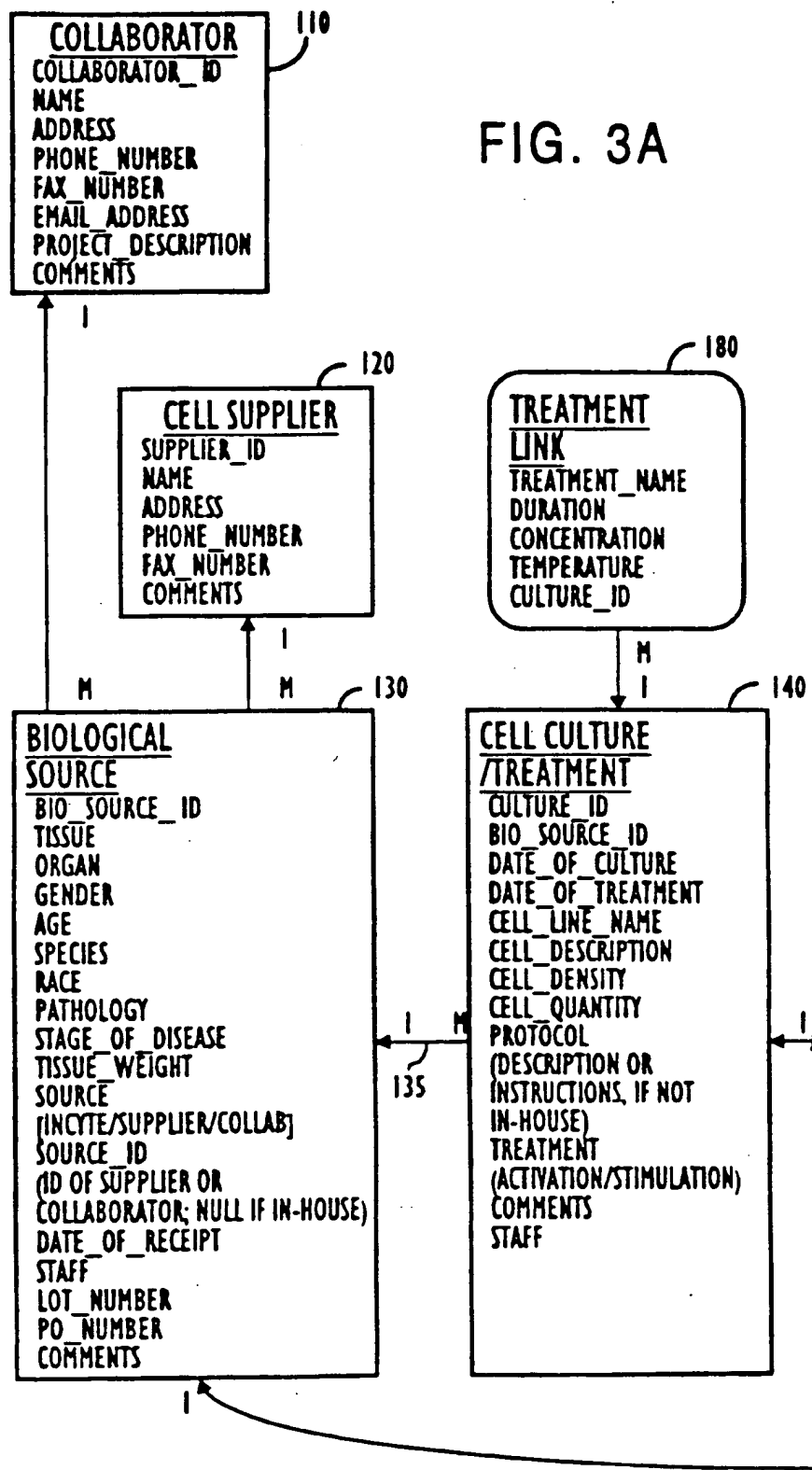


FIG. 3A



4/13

FIG. 3B

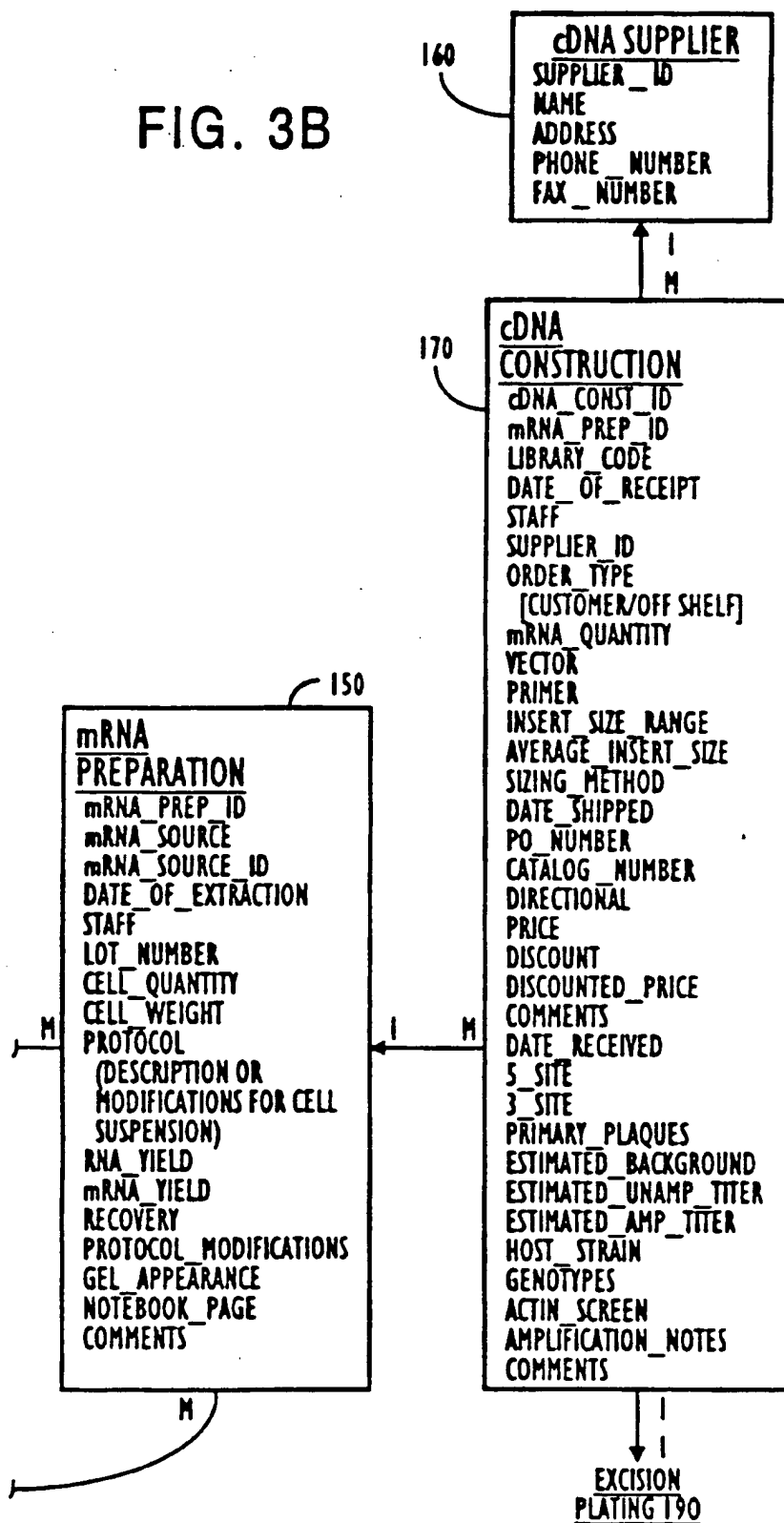


FIG. 4

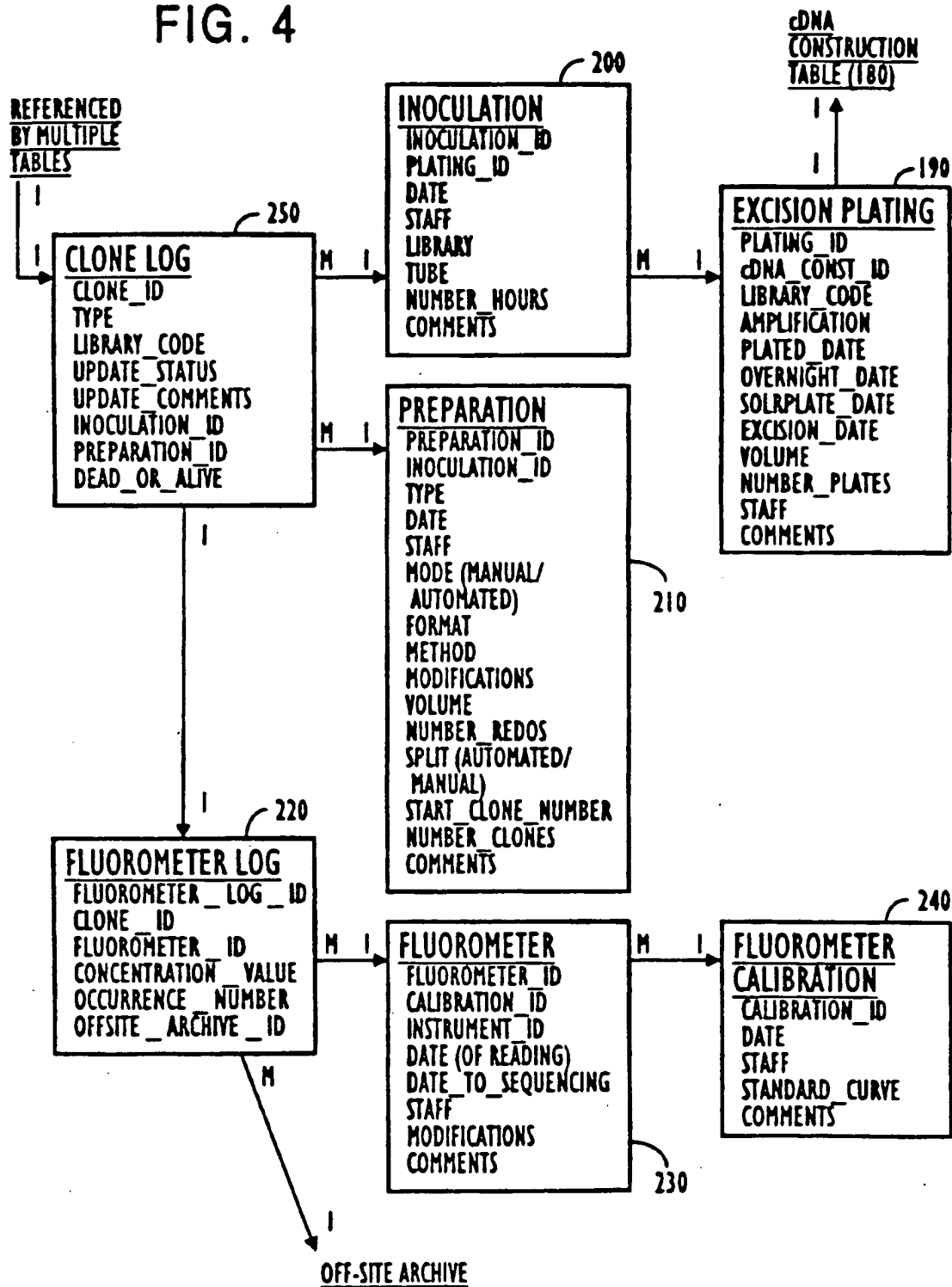


FIG. 5

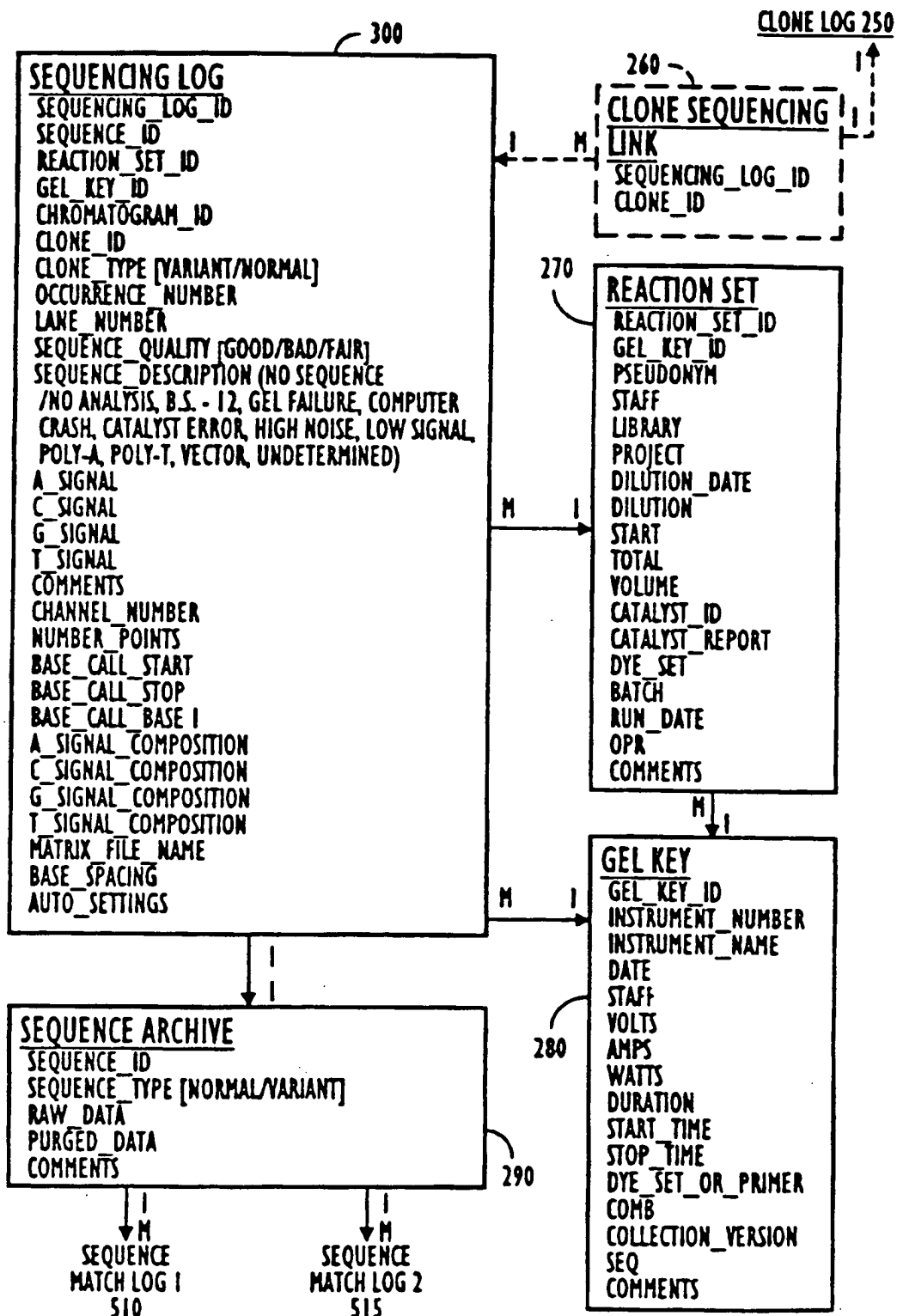


FIG. 6

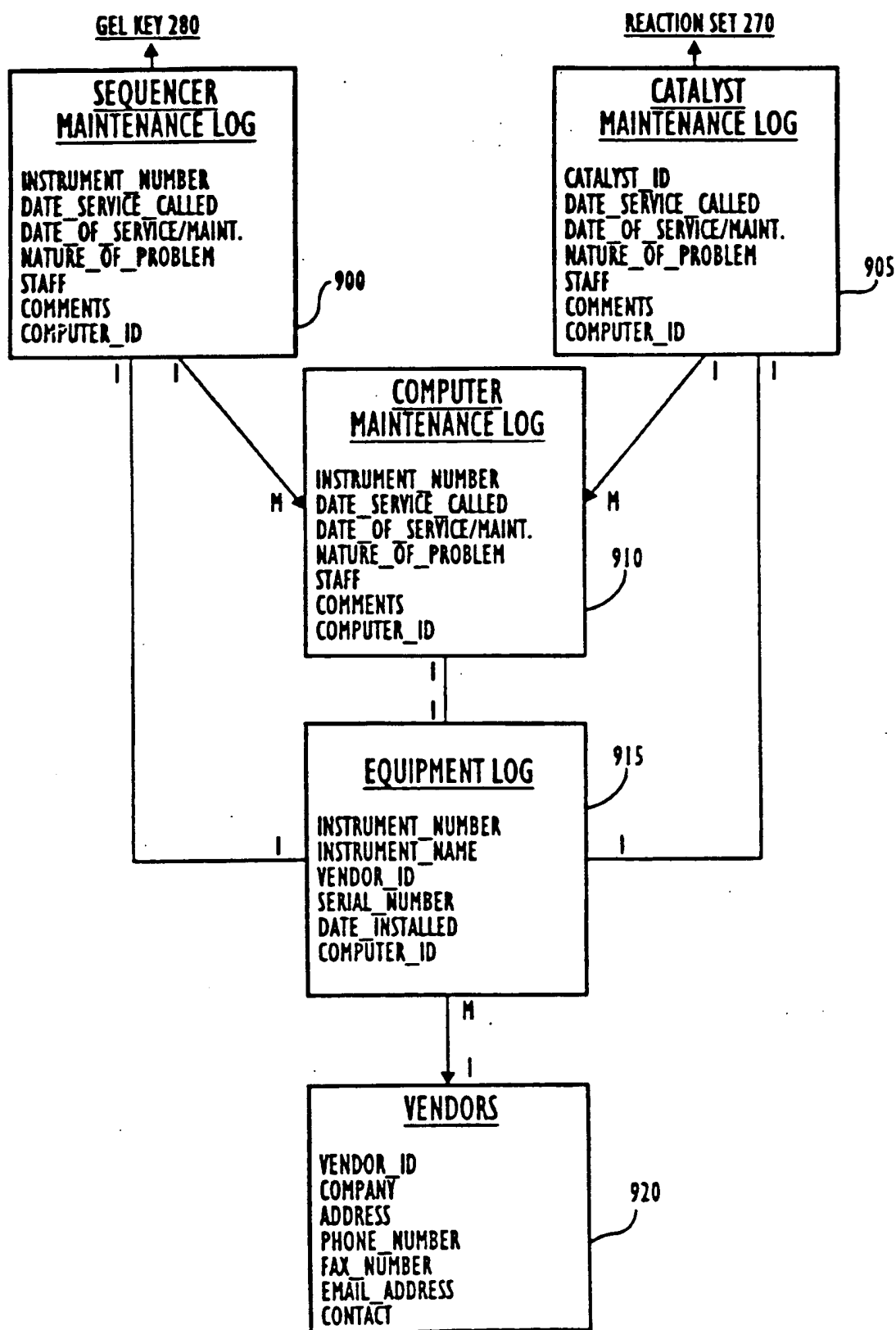
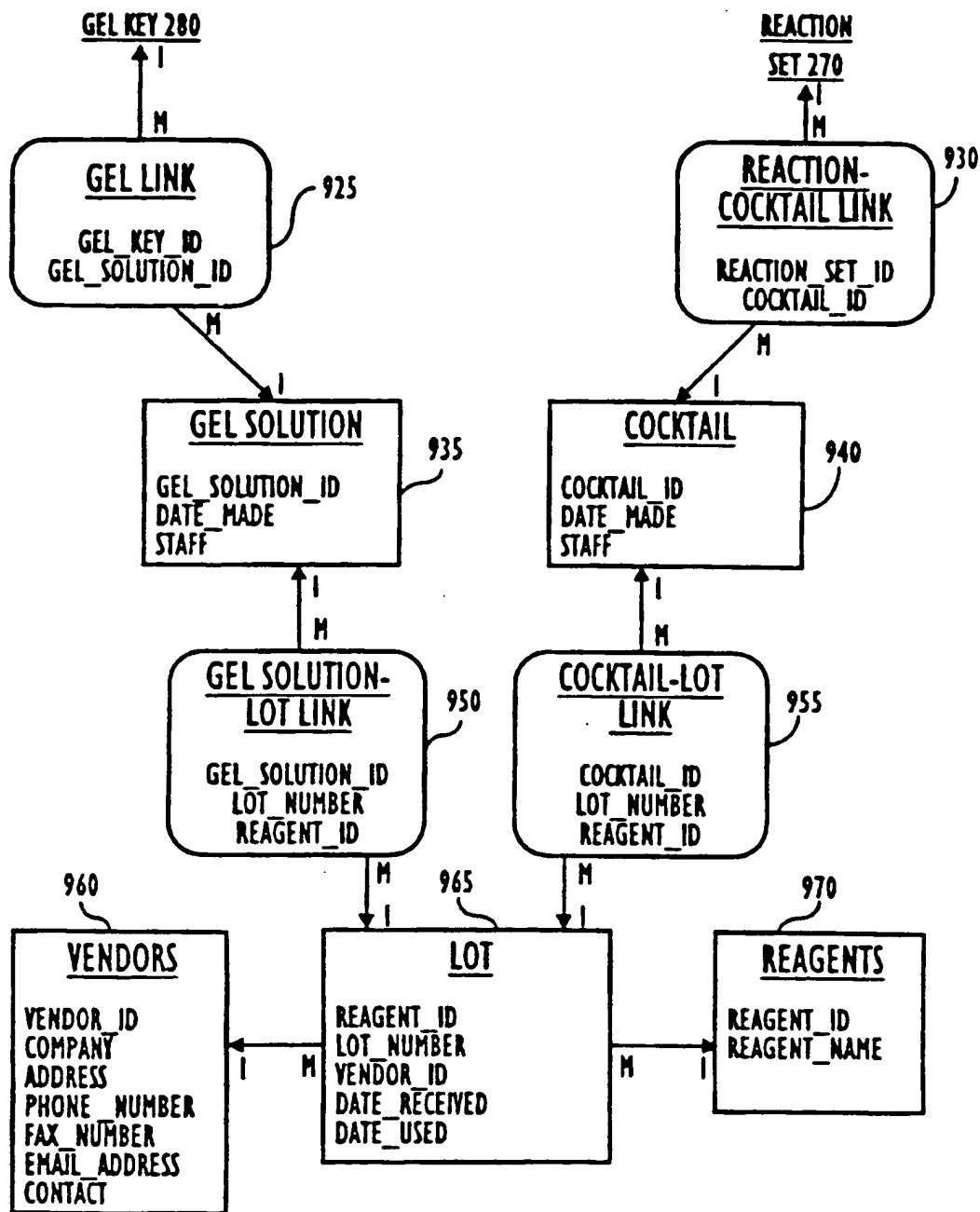
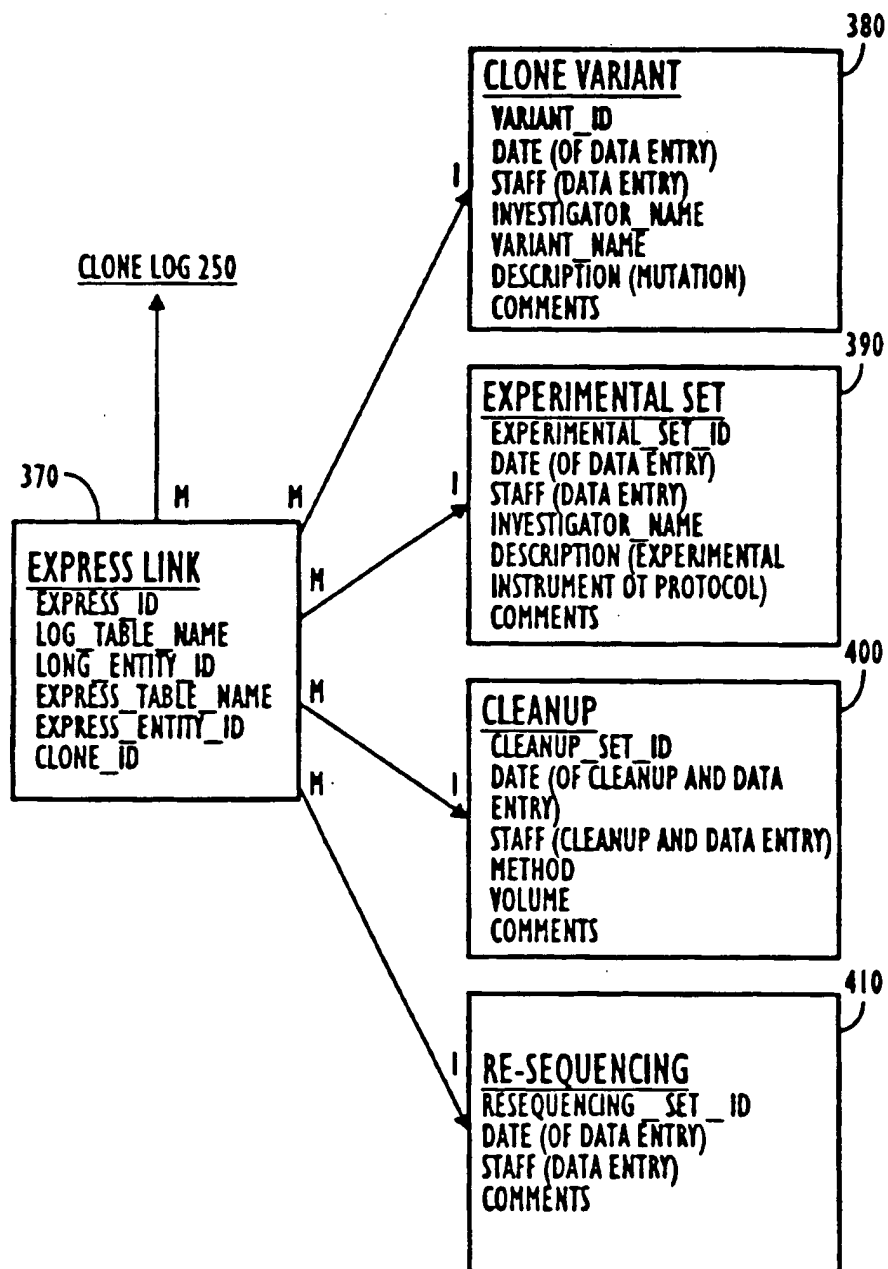


FIG. 7



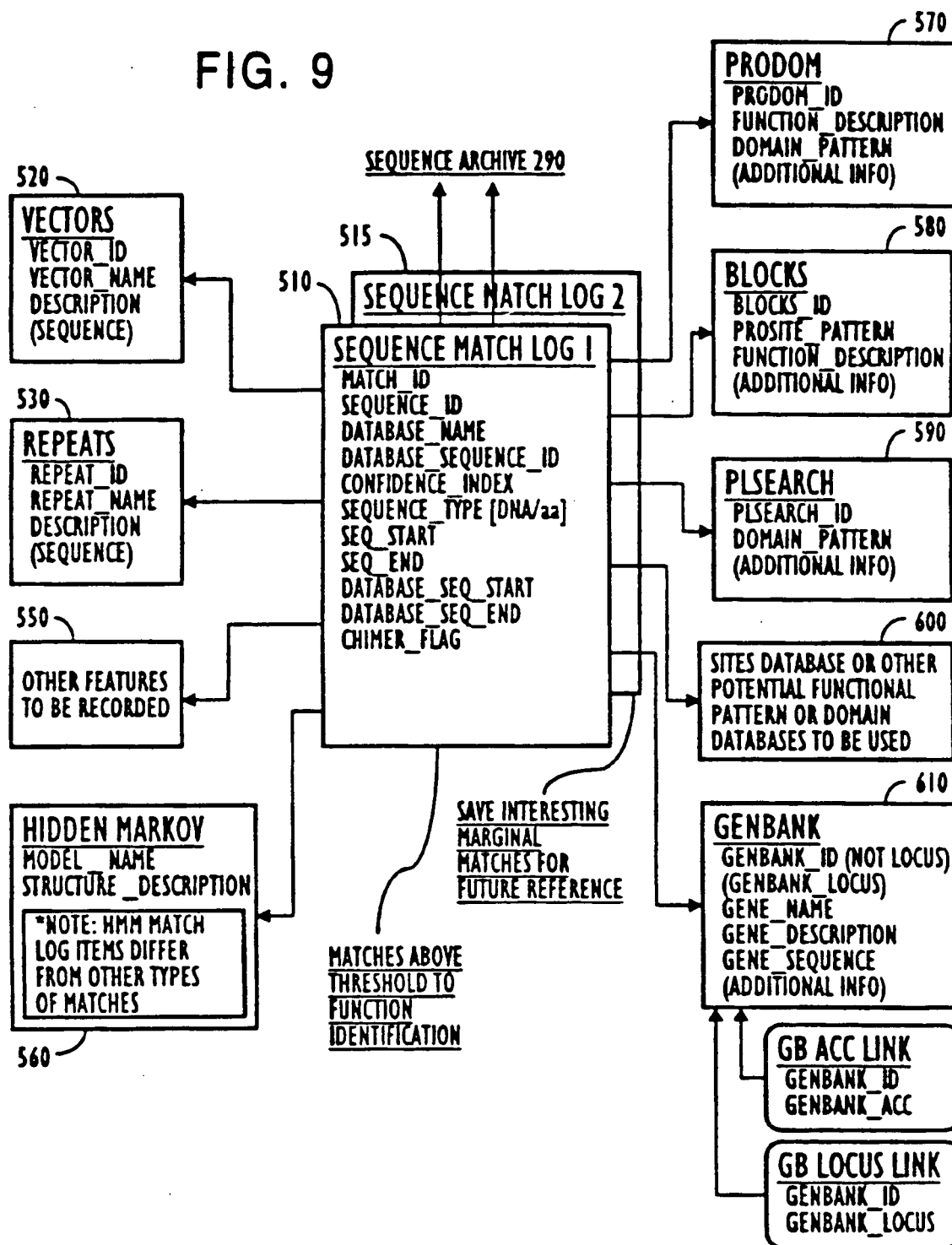
9/13

FIG. 8



10/13

FIG. 9



11/13

FIG. 10

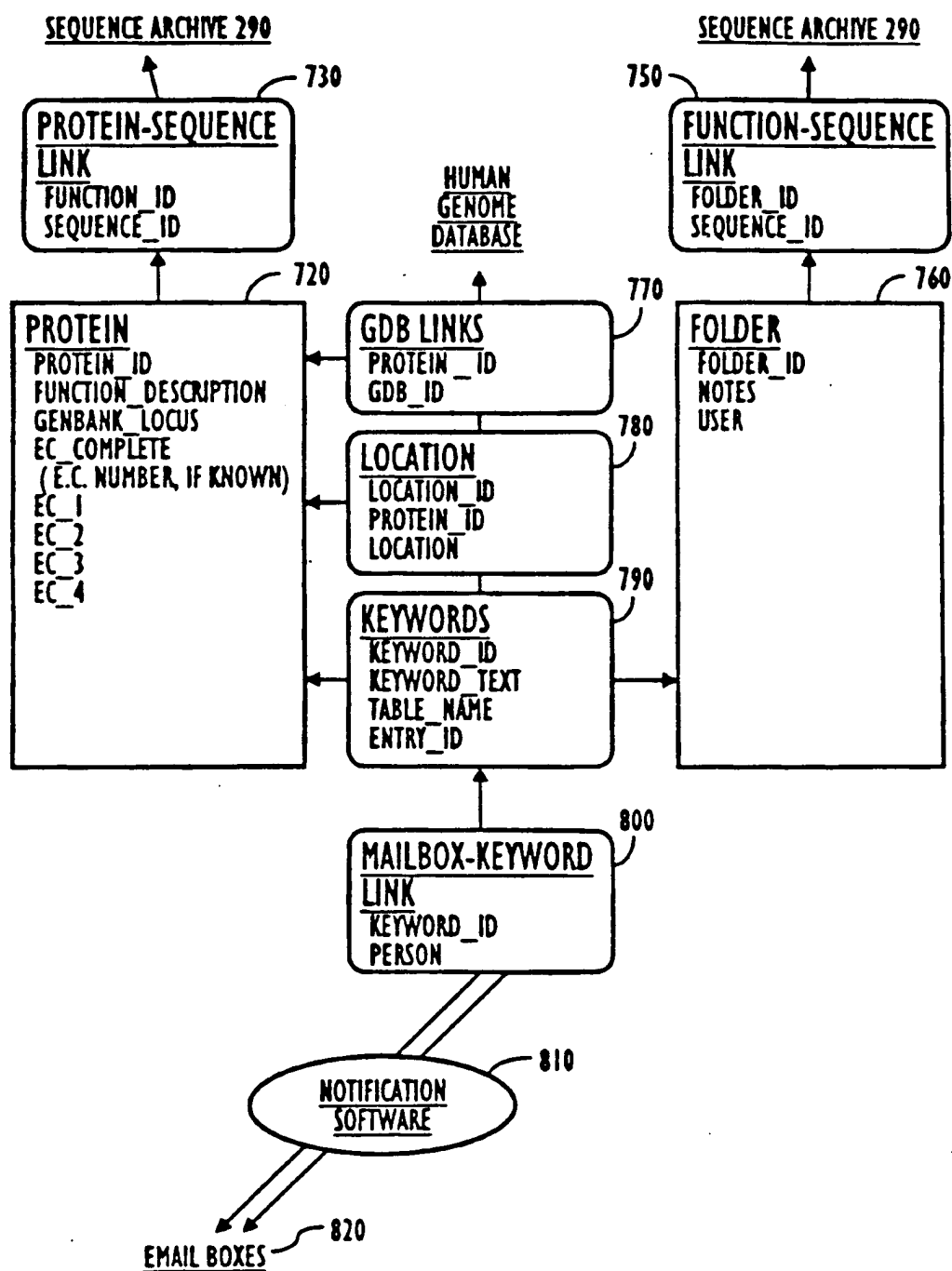


FIG. 11

1. INTERLEUKIN-1 BETA
2. MACROPHAGE INFLAMMATORY PROTEIN-1
3. INTERLEUKIN-8
4. LYMPHOCYTE ACTIVATION GENE
5. ELONGATION FACTOR-1 ALPHA
6. BETA ACTIN
7. RANTES T-CELL SPECIFIC PROTEIN
8. POLY A BINDING PROTEIN
9. OSTEOPONTIN; NEPHROPONTIN
10. TUMOR NECROSIS FACTOR-ALPHA
11. INCYTE CLONE 011050
12. Cu/Zn SUPEROXIDE DISMUTASE

FIG. 12

	<u>cDNA</u>	<u>TRANSCRIPT RATIO</u>
1.	INTERLEUKIN-1 BETA	262
2.	MACROPHAGE INFLAMMATORY PROTEIN-1	242
3.	INTERLEUKIN-8	238
4.	LYMPHOCYTE ACTIVATION GENE	142
5.	RANTES T-CELL SPECIFIC PROTEIN	46
6.	OSTEOPONTIN; NEPHROPONTIN	40
7.	INCYTE CLONE 011050	34
8.	TUMOR NECROSIS FACTOR-ALPHA	34
9.	Cu/Zn SUPEROXIDE DISMUTASE	28
10.	NGF-RELATED B CELL ACTIVATION MOLECULE	20
11.	IMMEDIATE EARLY RESPONSE PMA-IND GENE	18
12.	PROTEASE NEXIN-1, GLIAL-DERIVED	18
13.	INCYTE CLONE 011353	18
14.	INCYTE CLONE 010298	14
15.	INCYTE CLONE 011138	14

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/12429

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C12Q 1/68; (G06F 17/30 159:00)
US CL : 435/6; 364/413.1; 395/600

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6; 364/413.1; 395/600

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Science, Volume 252, issued 21 June 1991, M.D. Adams et al, "Complementary DNA sequencing: Expressed Swquence tags and human genome project", Pages 1651-1656, see entire document.	1-20
Y	Nucleic Acids Research, Volume 19, No. 25, issued 1991, E. Hara et al, "Subtractive cDNA cloning using oligo(dT)30-latex and PCR: isolation of cDNA clones specific to undifferentiated human embryonal carcinoma cells", pages 7097-7104, see entire document.	1-16, 18

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* "A"	Special categories of cited documents: document defining the general state of the art which is not considered to be part of particular relevance	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E"	earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reasons (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search

05 FEBRUARY 1996

Date of mailing of the international search report

20 FEB 1996

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

JACK M. CHOULES

Telephone No. (703) 305-9840

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/12429

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Mathematical methods for DNA Sequences, editor M. S. Waterman, copyright 1989, J. W. Frickett et al, "Development of a Database For Nucleotide Sequences", pages 2-34, especially pages 7-25.	1-20
X	Current Biology Ltd, 1993, K. Matsubara et al, "Identification of new genes by systematic analysis of cDNAs and database construction", pages 672-677, see entire document.	17-20
Y	Nature Genetics, Volume 2, issued November 1992, A. S. Khan et al, "Single pass sequencing and physical and genetic mapping of human brain cDNAs", pages 180-185,	1-16
A	US, A, 5,364,759 (CASKEY ET AL) 15 NOVEMBER 1994, see entire document	1-16
A	US, A, 5,371,671 (ANDERSEN ET AL) 06 December 1994, see entire document	1-16
A	Communications of the ACM, Volume 34, No. 11, issued November 1991, K. A. Frenkel, "The Human Genome Project and informatics: a monumental scientific adventure", pages 40-52, see entire document.	1-20

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US95/12429

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

APS, DIALOG, SPI, DR-LINK

search terms DNA, cDNA, RNA, mRNA, gene, genome, genetic, data, base, database, relational, frequency, concentration, location, tissue, organ, cell, cellular, structure, microbiology, clone, replicate, sequence